


Luo F, Zhang W, Meng Y et al. (2012) Identification of a side population (SP), which is characterized by high chemical efflux capacity, is present in human melanoma cell lines. *J Invest Dermatol* 132:1286–91

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van Steensel M, Steijlen PM, Bladegroen RS et al. (2005) A missense mutation in the type II hair keratin hHb3 is associated with monilethrix. *J Med Genet* 42:e19


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Beyond ABC: Another Mechanism of Drug Resistance in Melanoma Side Population

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It has been shown that a side population (SP), which is characterized by high chemical efflux capacity, is present in human melanoma cell lines. However, it was not clear whether patients’ samples contain the same subpopulation. In this issue, Luo et al. (2012) report that they have isolated SP cells directly from patients’ melanomas. SP cells are resistant to paclitaxel because of the upregulation of ABCB1 and ABCB5. Notably, these cells are also resistant to temozolomide, which is not a substrate for ATP-Binding Cassette (ABC) transporters, in an interleukin (IL)-8-dependent manner. This study provides novel clues for understanding how a small, but critical, subpopulation within melanomas is resistant to therapies. *The Journal of Investigative Dermatology* (2012) 132, 2317–2319. doi:10.1038/jid.2012.220

Melanomas are often resistant to pharmacological therapies. Conventional chemotherapy drugs currently used in clinics are of limited value in treating advanced melanomas. Tumor shrinkage induced by these drugs is often temporary, and most tumors progress or relapse after short periods of time. Both intrinsic and acquired mechanisms have roles in the chemoresistance of melanoma cells. One of the key properties for intrinsic resistance is the expression of certain ABC superfamily proteins, which function as ATP-dependent efflux transporters. The ABC family comprises nearly 50 members that are evolutionary highly conserved and have high sequence homology among its members. These transporters regulate tissue protection against endogenous and exogenous cellular toxins. Some of the ABC transporters are expressed ubiquitously among diverse cancer cell lines (NCI-60), whereas others are expressed selectively in cancer cells derived from particular tissue types (Szakacs et al., 2004). Melanoma cells express a group of ABC transporters, including ABCA9, ABCB1, ABCB5, ABCB8, ABCC2, and ABCD1 (Chen et al., 2009). Although it has been reported that ABCB5 and ABCB8

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Clinical Implications:

- Patient-derived melanoma tissues contain a side population (SP) that is chemotherapy-resistant.
- ATP-Binding Cassette (ABC) transporters ABCB1 and ABCB5 mediate resistance against paclitaxel.
- Resistance against temozolomide in SP cells is interleukin (IL)-8 dependent.

mediate doxorubicin resistance in melanoma cells (Frank et al., 2005; Elliott and Al-Hajj, 2009), and ABC2 expression is associated with cisplatin resistance (Liedert et al., 2003), the physiological functions of most ABC transporters in melanomas have not been elucidated.

The Hoechst 33342 dye exclusion assay is broadly performed to analyze the efflux capacity of cells. When Hoechst 33342 is excited by a UV laser, the dye emits blue (450 nm) and red (675 nm) wavelengths. A gradient of fluorescence of the dye monitored by flow cytometry at both wavelengths is usually seen as a comma-like region on an x-y plot. The tip of the comma-like region forms a tail, which displays low blue and red fluorescence. This subpopulation, termed ‘side population’, has high efflux capacity and has been identified in several tissues of mammalian species (Goodell et al., 1996). Likely, the increased efflux capacity of the SP is promoted through ABC transporters. Most commonly, ABCB1 and ABCG2 are highly expressed in SPs of many tissue types and are responsible for transporting Hoechst 33342, as well as chemotherapeutic drugs; vinblastin and paclitaxel are expelled by ABCB1, whereas topotecan and methotrexate are the substrates of ABCG2 (Badnagy et al., 2006).

The SP assay initially attracted attention among stem cell researchers as a strategy to isolate potential stem/progenitor cells from various tissues, because cell surface markers for stem cells are still not defined in many organ systems. More recently, the assay has been used with cell lines and patient-derived tumor material from various types of cancer to identify cells that exhibit stem cell-like properties. Several groups have characterized SP cells in melanoma (Grichnik et al., 2006; Fukunaga-Kalabis et al., 2010; Roesch et al., 2010; Wouters et al., 2012). Melanoma SPs from cell lines are small in size (Grichnik et al., 2006), slow growing (Grichnik et al., 2006; Roesch et al., 2010), and, most importantly, resistant to chemotherapy (Fukunaga-Kalabis et al., 2010; Wouters et al., 2012).

In this issue, Luo et al. (2012) report that patients’ melanomas indeed do contain an SP. They performed the Hoechst assay on suspensions of melanoma cells, which were directly isolated from tissue samples and patient-derived tumor xenografts (PDX model). Original tumors showed the presence of SP cells ranging from 0.1 to 0.7%, regardless of whether the lesions were primary or metastatic. Melanoma cell lines from previous and the current studies showed a heightened fraction of SP cells, close to 10% of the total population. It is likely that in vitro culture systems provide a selective pressure for the expansion of specific tumor subpopulations. PDX tumors showed a similar percentage of SP cells compared with their original tumor, indicating that this in vivo model has the significant advantage of maintaining the heterogeneity of the tumors of origin. Both paclitaxel and temozolomide treatment, the former is the substrate of ABCB1 and the latter is not, increased the ratio of SP cells in the remaining tumors of PDX mice, suggesting that SP cells in melanoma are resistant to chemotherapy in vivo. Melanoma SP cells showed higher expression of multiple ABC transporters, including ABCB1 and ABCB5, compared with the non-SP fraction. When these two ABC members were knocked down in a melanoma cell line, the percentage of the SP was significantly decreased, and the cells regained sensitivity to paclitaxel. Knocking down neither ABCB1 nor ABCB5 affected the response to temozolomide in melanoma. The authors further explored the mechanism responsible for temozolomide resistance of SP cells. The patient-derived SP cells highly expressed IL-8, which has been suggested to be associated with chemoresistance in melanoma cells. Blocking IL-8 signaling with neutralizing antibodies or small interfering RNA significantly increased sensitivity to temozolomide in vitro (the former specifically decreased the resistance in a SP). Moreover, microarray data revealed that SP cells are enriched with components of the inflammatory response, especially in the NF-kB signaling pathway, which may contribute to chemoresistance in an ABC transporter-independent manner. These findings suggest that not only efflux capacity, but also other intrinsic mechanisms confer the resistance of melanoma SP cells against multiple drugs, which are not substrates for ABC transporters. Further studies are warranted to determine whether the inhibition of IL-8 or NF-kB signaling can reverse drug resistance of melanoma cells in vivo. Such studies will contribute to progress in the therapy of this tumor, which remains challenging to treat.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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REFERENCES


Hadnagy A, Gaboury L, Beaulieu R et al. (2006) SP analysis may be used to identify cancer stem cell populations. Exp Cell Res 312:3701–10